American Journal of Medical Genetics (Neuropsychiatric Genetics) 74:319–323 (1997)

Linkage Study of Chromosome 6p in Sib-Pairs With Schizophrenia

J.K. Daniels,[1,2] G. Spurlock,[1,2] N.M. Williams,[1,2] A.G. Cardno,[1] L.A. Jones,[1] K.C. Murphy,[1] P. Asherson,[1,2] P. Holmans,[1] I. Fenton,[2] P. McGuffin,[1] and M.J. Owen[1,2]*

[1]Department of Psychological Medicine, University of Wales College of Medicine, Cardiff, United Kingdom
[2]Department of Medical Genetics, University of Wales College of Medicine, Cardiff, United Kingdom

Following reports of linkage between schizophrenia and markers in the chromosomal region 6p24-22 we have studied nine microsatellite markers spanning 40 cM of this region in our sample of 102 affected sibling pairs from 86 families. Allele sharing identity by descent was examined using likelihood based sib-pair analysis as implemented by the program SPLINK. No evidence for linkage was obtained and the highest lod score was only 0.192 for D6S309. We conclude that if there is a susceptibility locus for schizophrenia in this region then its effect size is so small as to render our study insufficiently powerful to detect it. Am. J. Med. Genet. 74:319–323, 1997.

KEY WORDS: schizophrenia; sibling pairs; allele sharing; linkage; chromosome 6

INTRODUCTION

Evidence for an important genetic contribution to schizophrenia comes from family, twin, and adoption studies. However the mode of inheritance, at least in the great majority of cases, is complex and non-mendelian [McGuffin et al., 1995]. Up until recently, studies of genetic markers in multiply affected families have failed to result in replicated linkages. However, a group from the Medical College of Virginia (MCV) have recently reported evidence for linkage to markers from the chromosomal region 6p24-22 in a large sample of 256 families [Straub et al., 1995; Wang et al., 1995]. A maximum lod score (Z_{max}) of 3.51 was found at D6S296. Estimates of heterogeneity suggested that the disease allele was segregating in approximately 15–30% of families, although the results were also consistent with oligogenic inheritance. Suggestive, but not significant, linkages to markers in the same region were subsequently reported by three independent groups [Moises et al., 1995; Schwab et al., 1995; Antonarakis et al., 1995] but not by others [Mowry et al., 1995; Gurling et al., 1995]. A summary of these studies is shown in Table I.

In view of these findings we have studied nine markers spanning a 40 cM region of the short arm of chromosome 6 in 102 affected sib-pairs with schizophrenia.

MATERIALS AND METHOD

Sample

Families in which two or more siblings met the criteria for DSM IV schizophrenia or schizoaffective disorder (American Psychiatric Association, 1994) were ascertained through mental health services and relatives' support groups in England and Wales. The sample consisted of 180 affected individuals from 86 families grouped as 78 pairs and 8 triplets; 126 subjects were male and 54 were female. All subjects were Caucasian and had been born in the UK. Mean age of the sample was 43.1 years (s.d. 12.3) and mean duration of illness was 18.3 years (s.d. 12.9).

Diagnostic Process

All patients underwent a semi-structured interview, which was conducted by a trained member of the research clinical assessment team who was either a psychiatrist or a psychologist [PSE 9: Wing et al., 1974; or SCAN: Wing et al., 1990]. Diagnoses were based on all available clinical information including an examination of case records and information from relatives and mental health professionals. These data were compiled into case vignettes for each subject. Diagnoses were made independently by the interviewer and another member of the clinical assessment team. If the diagnoses agreed this became the consensus diagnosis. In cases of disagreement the third member of the team made a further independent diagnosis, and the case was discussed in detail in order to reach consensus. Inter-rater reliability was assessed on 40 cases with a variety of functional psychoses. All three members of the clinical assessment team made independent diagnoses, and these were compared with consensus diag-

*Correspondence: M.J. Owen, Department of Psychological Medicine, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, United Kingdom. Email: wpcmjo@cardiff.ac.uk

Received 30 September 1996; Revised 14 January 1997

TABLE I. Published Studies of 6p Linkage and Schizophrenia

Investigator	Sample	Highest lod score at; (zmax)	Diagnosis classification	Mode of inheritance
Wang et al.	186 multiplex families	D6S260; (3.2)	Broad	Dominant
Straub et al.	Enlarged sample of Wang et al. 265 multiplex families	D6S296; (3.51)	Broad	Dominant
Schwab et al.	43 families	D6S274 (2.2)	Narrow	Dominant
Moises et al.	5 large pedigrees 65 multiplex families 113 trios	D6S274 (0.005)*	Narrow	Dominant
Antonarakis et al.	57 families	D6S296; (1.17)	Narrow	Recessive
Mowry et al.	45 families	D6S259; (0.34)	Narrow	Recessive
Gurling et al.	23 families	D6S285; (0.214)	Narrow	Recessive

*P value for second stage replication.

noses. Kappa coefficients ranged from 0.85 to 0.94. Ongoing monitoring of clinical assessment standards was carried out by means of weekly meetings in which each team member made assessments of prepared case vignettes, audiotapes and videotapes of interviews.

160 subjects received a DSM IV diagnosis of schizophrenia, 10 were diagnosed as schizoaffective disorder depressive type, and 10 were diagnosed as schizoaffective disorder bipolar type.

Genotyping

High molecular weight DNA was extracted from lymphocytes according to routine procedures. Nine microsatellite markers from the Genethon linkage map [Dib et al., 1996] were typed. Intermarker distances are shown in Table II. Six of the markers were amplified with radioactively labelled (^{33}P) 5′-primers. Subsequent sizes of the alleles were determined by comparison to M13mp18 sequencing ladders on 6% denaturing polyacrylamide gels. The remaining three markers were amplified using fluorescently labelled 5′-primers. Allele calling was determined by analysis with Genescan™ and Genotyper™ software by comparison of the fragment sizes to an internal standard. All individuals were genotyped by two independent raters.

Statistical Analysis

Sib pairs obtained from trios were weighted by 2/3 [Suarez et al., 1979]. Single-locus affected sib pair analyses were performed using the program SPLINK [Holmans and Clayton, 1995]. This implements the likelihood-ratio method originally proposed by Risch [1990]. The likelihood of the marker data from each nuclear family is expressed as a linear combination of the (unknown) ibd sharing probabilities of the affected pair. The likelihood of the whole sample is maximised with respect to these probabilities, subject to the constraint that they correspond to a possible genetic model [Holmans, 1993]. A likelihood-ratio test is performed by comparing the maximised likelihood to its value under the null hypothesis of no linkage. Note that, unless both parents are typed, the likelihood of each nuclear family will depend on the marker allele frequencies. SPLINK estimates these from the sample, reducing the chance of false-positive results brought about by the use of inappropriate allele frequency estimates. Multipoint analyses, including exclusion mapping, were performed using the MAPMAKER/SIBS package [Kruglyak and Lander, 1995]. We also subjected the data to both single locus and multipoint parametric analyses using GENEHUNTER [Kruglyak et al., 1996]. Three

TABLE II. Summary of Result Obtained by Analysis of Allele Sharing Identity by Descent in Sibling Pairs*

	Marker	NF	NI	Pr (0 ibd)	Pr (1 ibd)	Pr (2 ibd)	Lod score
	D6S309	80	48.99	0.23	0.46	0.31	0.192
1 cM							
	D6S296	74	45.41	0.25	0.50	0.25	0
4 cM							
	D6S470	75	42.63	0.24	0.48	0.28	0.068
9 cM							
	D6S259	70	32.55	0.20	0.50	0.30	0.162
4 cM							
	D6S260	84	61.49	0.22	0.48	0.30	0.185
4 cM							
	D6S285	80	37.19	0.24	0.50	0.26	0.009
7 cM							
	D6S461	84	32.51	0.24	0.50	0.26	0.010
6 cM							
	D6S276	81	47.97	0.25	0.50	0.25	0
5 cM							
	D6S291	86	33.83	0.23	0.47	0.30	0.111
Centromere							

*Genetic distances were taken from the Genethon map [Dib et al. 1996]. NF, no. of families analysed; NI, equivalent number of fully informative sib pairs; D6S285 is 1.3 cM proximal to D6S274.

genetic models were tested corresponding to DOM, REC, and PEN of Straub and colleagues [1995].

RESULTS

Results are summarized in Table II. No evidence for linkage was obtained for any marker in this 40 cM region of 6p (All lod score P values > 0.1). The highest lod score was obtained for D6S309 ($z_{max} = 0.192$). Figure 1 shows the results of multipoint analysis across the 40 cM region. A maximum lod score of 0.225 was obtained at D6S260. Multipoint exclusion mapping was carried out assuming that the putative 6p locus contributes a relative risk to sibs (λ_S) of either 1.2, 1.5, 2, or 3 (Fig. 2). It can be seen that a locus with a $\lambda_S = 3$ can be excluded from almost the entire region. A locus with a $\lambda_S = 2$ can only be excluded from a small region centromeric of D6S461. It is not possible to exclude a locus with $\lambda_S = 1.5$ or 1.2 from anywhere within the region.

Parametric analyses also failed to reveal evidence for linkage. For the intermediate model (PEN) of Straub and colleague [1995] the largest single locus hlod was 0.16 at D6S260 ($\theta = 0.01$, $\alpha = 0.15$). Multipoint analysis with the PEN genetic model resulted in a maximum hlod of 0.13 at D6S309 ($\lambda = 0.133$). Results with the dominant model (DOM) were similar. With the recessive model (REC) the largest single locus hlod was 0.23 at D6S309 ($\theta = 0$, $\alpha = 0.1$) and the maximum multipoint hlod was 0.21 ($\alpha = 0.1$) midway between D6S470 and D6S259).

DISCUSSION

We were unable to obtain evidence for linkage in the region of chromosome 6p implicated by the MCV group [Straub et al., 1995; Wang et al., 1995] in spite of the suggestive findings of others studying markers in the same region [Moises et al., 1995; Schwab et al., 1995; Antonarakis et al., 1995]. It seems unlikely that this was due to the study of affected sib-pairs rather than multiply affected families since the majority of the kindreds studied by the MCV group and all of those studied by Schwab and colleagues were nuclear pedigrees. It is also unlikely to have been due to differences in markers studied since we used markers across the whole putatively linked region and included those loci yielding the maximum lod scores in all four studies supporting linkage to 6p (D6S296, D6S260, and D6S285). Finally, it seems unlikely that the differences between our results and those of the MCV group are due to analytic differences since we were unable to demonstrate linkage even when we used the same genetic parameters (PEN model) that yielded the highest lod scores in the MCV study.

Recent data from a large, multicentre collaborative analysis of 6p markers in schizophrenia [Schizophrenia Collaborative Linkage Group for Chromosomes 3, 6,

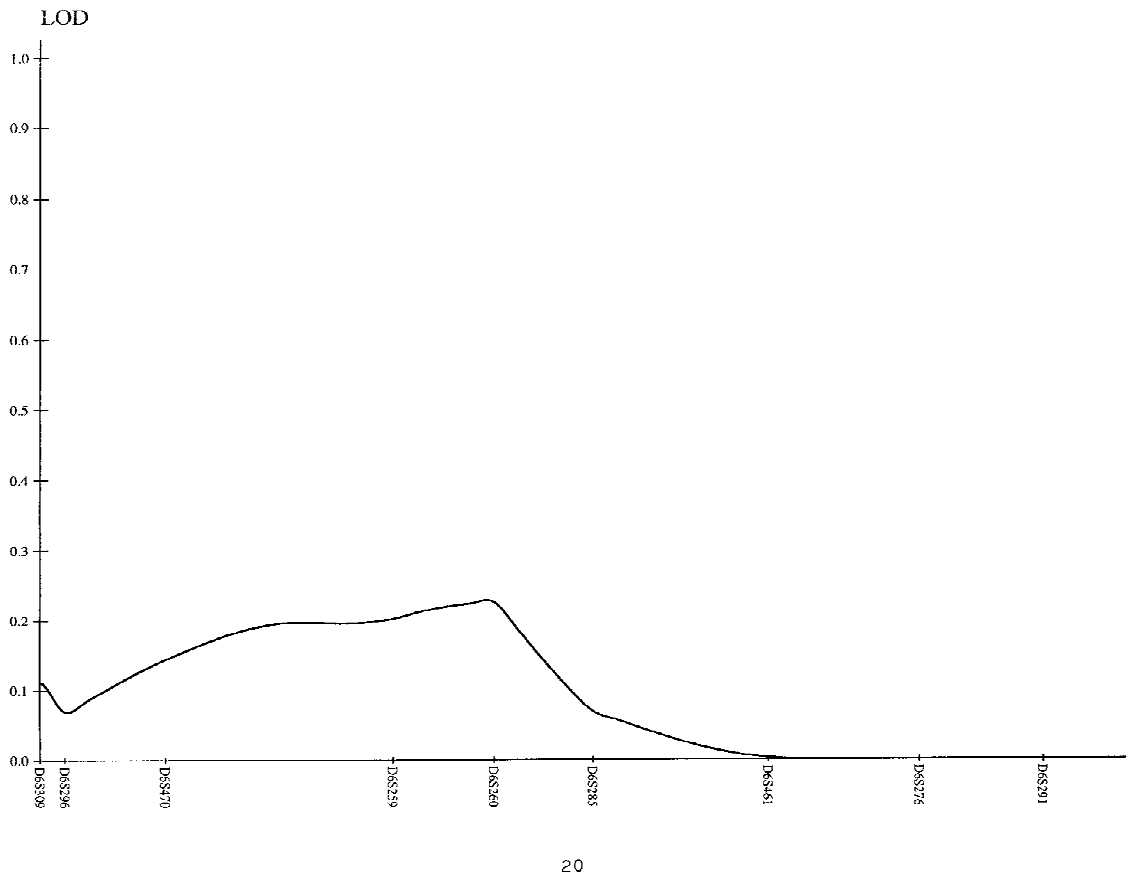

Fig. 1. Graph of multipoint maximum lod score.

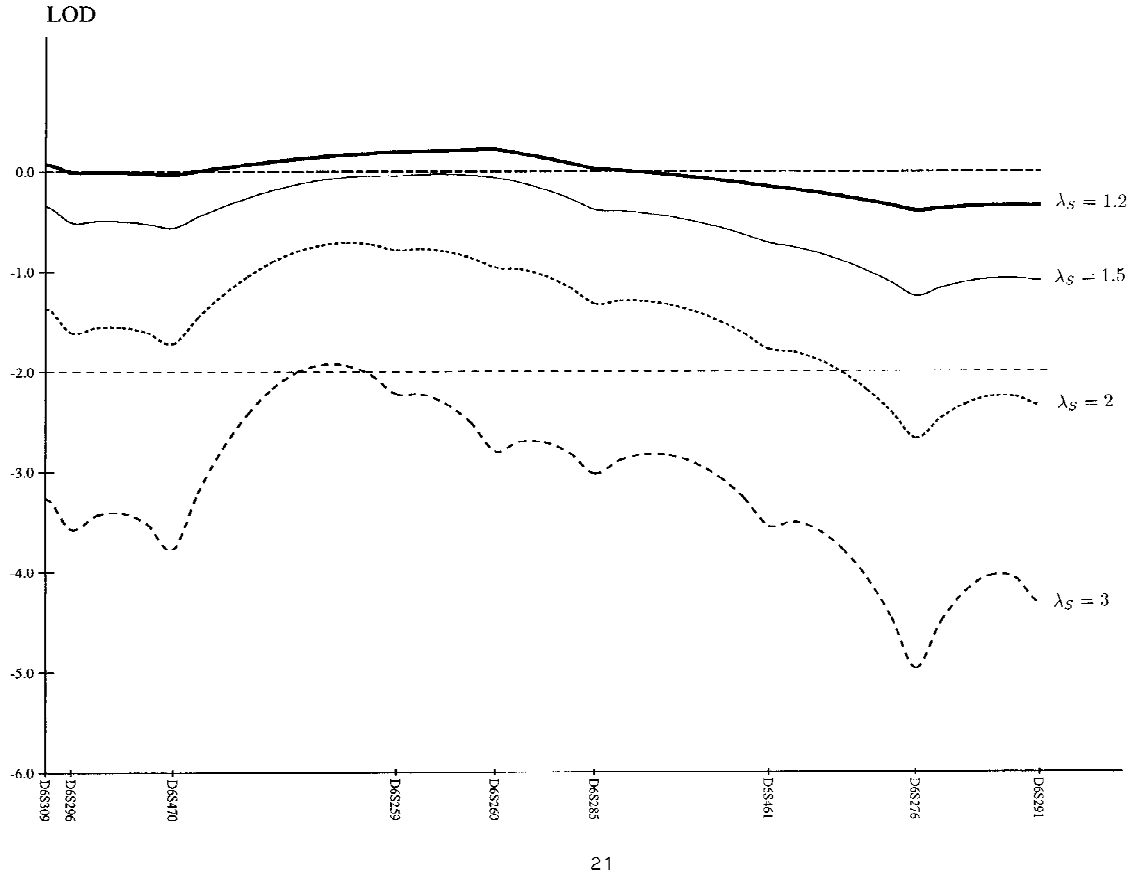

Fig. 2. Graph of multipoint exclusion lod scores for $\lambda_S = 1.2$, $\lambda_S = 1.5$, $\lambda_S = 2$, and $\lambda_S = 3$.

and 8, 1996] suggest that the failure of ourselves and others [Mowry et al., 1995; Gurling et al., 1995] to replicate the MCV findings could be due to lack of power resulting from the small effect size of the locus on 6p. The collaborative analysis contained 448 pedigrees yielding 367 sibships with 2 or more affected and included the positive data from Schwab et al. [1995] and Antonarakis et al. [1995] as well as those from many of the centres contributing to the collaborative study reported by Moises et al. [1995]. Equivalent numbers of fully-informative sib pairs ranged from 200 to 332 for each of the five markers from 6p (D6S296, D6S277, D6S470, D6S259, and D6S285). The data were analysed by both non-parametric and parametric methods. The maximum lod score obtained by multipoint sib-pair analysis was 2.19 between D6S470 and D6S259. This corresponds to a very small genetic effect, with the relative risk to sibs contributed by the putative 6p locus (λ_S) of 1.25. The maximum lod score under parametric analysis was only 1.22 at D6S277 for a recessive model. We have calculated that the sample used in the present study, while large in comparison with many reported by individual groups to date, had a power of only 0.244 to detect linkage to a locus of this effect size at a significance level of 0.05 . Furthermore we were unable to exclude a locus contributing a $\lambda_S < 2$ from anywhere within the region studied.

The MCV group found that the evidence for linkage in this region increased substantially when the diagnostic model included a broader range of phenotypes. This did not occur in the collaborative study described above. We were unable to examine this issue in the present study as our sample collection strategy focused only upon narrowly defined cases (i.e., those with schizophrenia or schizoaffective disorder).

It remains possible that some of the differences between studies reflect heterogeneity and that the 6p locus is more important in some geographical populations such as the Irish one from which the MCV sample was obtained. However, considerable difficulties in interpreting the positive findings remain. A very large number of families have now been studied and the results are at best only highly suggestive of a susceptibility locus for schizophrenia at 6p22-24. The possibility that this represents a false positive cannot be excluded. The putatively linked region extends over approximately 30 cM and it seems unlikely that future linkage studies will have sufficient power either to confirm linkage unambiguously or to refine the position of the disease gene with sufficient precision to allow positional cloning.

These problems could be overcome if linkage disequilibrium (LD) were to be detected between a marker or markers in this region and the disease, and allelic association studies with 6p markers are now a priority. If LD cannot be detected then positional cloning of the disease gene is unlikely. In this case we shall have to rely upon the generation and testing by mutation

analysis of possible candidate genes within the region. Moreover, this arduous endeavour would have to take place in the face of continuing uncertainty as to the existence of a susceptibility locus in this region.

ACKNOWLEDGMENTS

This work was supported by a program grant from the Medical Research Council (UK) and by a grant from Glaxo Research and Development. We would like to thank Genethon for providing us with information regarding markers on 6p from the new Genethon human genetic linkage map prior to publication

REFERENCES

Antonarakis SE, Blouin J-L, Pulver AE, Wolyniec P, Lasseter VK, Nestadt G, Kasch L, Kazazian HH, Dombroski B, Kimberland M, Ott J, Housman D, Karayiorgou M, Maclean CJ (1995): Schizophrenia susceptibility and chromosome 6p24-22. Nat Genet 11:235–236.

Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J (1996): A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154.

Gurling H, Kalsi G, Hui-Sui Chen A, Green M, Butler R, Read T, Murphy P, Curtis D, Sharma T, Petursson H, Brynjolfsson J (1995): Schizophrenia susceptibility and chromosome 6p24-22. Nat Genet 11:234–235.

Holmans P (1993): Asymptotic properties of affected sib pair linkage analysis. Am J Hum Genet 52:362–374.

Holmans P, Clayton D (1995): Efficiency of typing unaffected relatives in an affected sib-pair linkage study with single-locus and multiple tightly-linked markers. Am J Hum Genet 57:1221–1232.

Kruglyak L, Lander E (1995): Complete multipoint sib pair analysis of qualitative and quantitative traits. Am J Hum Genet 57:439–454.

Kruglyak L, Daly MJ, Reeve-Daly MR, Lander ES (1996): Parametric and non-parametric analysis: A unified multipoint approach. Am J Hum Genet 58:1347–1363.

McGuffin P, Owen MJ, Farmer AE (1995): The genetic basis of schizophrenia. Lancet 346:678–682.

Moises HW, Yang L, Kristbjarnarson H, Wiese C, Byerley W, Macciardi F, Arolt V, Blackwood D, Liu X, Sjogren B, Aschauer HN, Hwu H-G, Jang K, Livesley WJ, Kennedy JL, Zoega T, Ivarsson O, Bi M-T, Yu M-H, Havsteen B, Commenges D, Weissenbach J, Schwinger E, Gottesman II, Pakstis AJ, Wetterburg L, Kidd KK, Helgason T (1995): An International genome-wide search for schizophrenia susceptibility genes. Nat Genet 11:321–324.

Mowry BJ, Nancarrow DJ, Lennon DP, Sandkuijl LA, Crowe RR, Silverman JM, Mohs RC, Siever LJ, Endicott J, Sharpe L, Walters MK, Hayward NK, Levinson DF (1995): Schizophrenia susceptibility and chromosome 6p24-22. Nat Genet 11:233–234.

Risch N (1990): Linkage strategies for genetically complex traits. III. The effect of marker polymorphism on analysis of affected relative pairs. Am J Hum Genet 46:242–253.

Schizophrenia Collaborative Linkage Group for Chromosomes 3, 6 and 8 (1996): Additional support for schizophrenia linkage findings on chromosomes 6 and 8. Am J Med Genet 67:580–594.

Schwab SG, Albus M, Hallmayer J, Hoing S, Bormann M, Lichtermann D, Ebstein RP, Ackenheil M, Lerer B, Risch N, Maier W, Wildemauer DB (1995): Evaluation of a susceptibility gene for schizophrenia on chromosome 6p by multipoint affected sib-pair linkage analysis. Nat Genet 11:325–327.

Straub ER, Maclean CJ, O'Neill A, Burke J, Murphy B, Duke F, Skinkwin R, Webb BT, Zhang J, Walsh D, Kendler KS (1995): A potential vulnerability locus for schizophrenia on chromosome 6p24-22: evidence for genetic heterogeneity. Nat Genet 11:287–292.

Suarez BK, Hodge S (1979): A simple method to detect linkage for rare recessive diseases: An application to juvenile diabetes. Clin Genet 15: 126–136.

Wang S, Sun C, Walczak CA, Ziegle JS, Kipps BR, Goldin LR, Diehl SR (1995): Evidence for a susceptibility locus for schizophrenia on chromosome 6pter-p22. Nat Genet 10:41–45.

Wing JK, Babor T, Brugha T (1990): SCAN: Schedules for the clinical assessment in neuropsychiatry. Arch Gen Psychiatry 47:589–593.

Wing JK, Cooper JE, Satorius N (1974): "The Measurement and Classification of Psychiatric Illness." Cambridge: Cambridge University Press.